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PRODUCTION OF BEERS WITH THE ADDITION OF FUNCTIONAL PLANTS

– Research paper –

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Abstract: As the beer market diversifies, novel types of beers are appearing. An interesting possibility is the use of functional plant materials, which may improve physicochemical properties of beers and contribute to creating a beverage with intriguing sensory qualities. The aim of this study was to evaluate the contribution of selected plant additives to the physicochemical and sensory profile of beer. The study was conducted on brewers' wort with aniseed fruit, rosehip, cistus, moringa oleifera, Jerusalem artichoke, and ashwagandha at two doses (0.1% and 0.2%). A beer without any additions was used as a control sample. The weight changes of beers during fermentation were analyzed. Alcohol content, real extract, free amino nitrogen, titratable acidity, color, pH, and sensory analysis were performed on the produced beers. The highest alcohol content was obtained in beers with Jerusalem artichoke (0.2%), while the lowest was found in a beverage with ashwagandha (0.2%). Beers with evaluated ingredients were characterized by a lower titratable acidity, compared to the control sample. The sensory analysis revealed that the beers with Jerusalem artichoke (0.2%) and moringa leaves (0.2%) found the greatest approval among panelists.

Keywords: beer, ashwagandha, moringa leaves, aniseed, Jerusalem artichoke, sensory analysis

INTRODUCTION

With the rising consumer awareness of the health-promoting properties of food, products described as functional foods have begun to appear on the market. In the legal framework of the European Union, a definition of these foods has been created by The European Commission Concerted Action on Functional Food Science in Europe (FUFOSE). According to that definition, functional food is: "a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. It is consumed as part of a normal food pattern. It is not a pill, a capsule, or any form of dietary supplement" (Harasym et al., 2016). In such a context, beer, due to its composition and beneficial effects on the human body, can be used as a basis for developing valuable products (Stachowiak and Bukowski, 2021).

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Nowadays, consumers are increasingly interested in products with unique taste qualities, fermented products with lower alcohol content, and those whose ingredients show health-promoting effects. To meet the above expectations, craft beer experts are producing, among other products, non-alcoholic and reduced alcohol, gluten-free or isotonic versions of this beverage (Borșa et al., 2022). Some of the most popular plants added to a beer include ginger root, lavender, juniper, or coriander. Herbs and spices such as lemongrass, heather, chili peppers, orange and lemon peels, anise, and licorice are also widely used. Fruit juices and concentrates, e.g., from raspberries, chokeberries, and cornelian cherries are other popular ingredients. These ingredients are known to have a number of health-promoting benefits, and unique sensory qualities (Stachowiak and Bukowski, 2021).

The plants used in this study are commonly used in many industries (cosmetic, pharmaceutical, and food). In addition to their nutritional and technological roles, they exhibit health-promoting properties. However, proving the functionality of a product for a specific health condition is challenging, and obtaining approval from the relevant authorities can often be difficult. Therefore, adding any specific health claims to produced products must be avoided unless thorough clinical evidence is available, and in the case of Europe, such claims must be approved by EFSA (Alongi and Anese, 2021). Functional food is defined in various ways, but according to Kaur and Das (Kaur and Das, 2011), foods can be named as functional, if they belong to at least one of the categories: (a) fortified with ingredients that have a positive effect on health, (b) processed to counteract anti-nutritional compounds, (c) novel foods with proven health effects, or (d) raw materials enhanced or fortified through changes in agricultural practices or through post-harvest treatments applied to fruits and vegetables.

In such approach, beers produced with functional ingredients would fall into the first of the aforementioned categories. According to Zeng et al. (Zeng et al., 2024) beer itself can be seen as a functional product, due to a high concentration of health-promoting compounds, such as polyphenols, melatonin, minerals or bitter acids. In this case, the addition of materials with known health benefits (as those used in the study), can further improve functional characteristics of a beer, if such substances are extracted into a beer.

Anise, similar to caraway and fennel, is a spasmolytic agent and is helpful in digestive and other gastric ailments. Anise seed and its essential oil exhibit antioxidant, antifungal, anticonvulsant, anti-inflammatory, analgesic, antidiabetic, and antiviral effects. Aniseed water extract exhibits antioxidant activity (Mohammad Al-Ismail and Aburjai, 2004; Sun et al., 2019).

Rosa canina L. fruits show preventive and therapeutic effects against arthritis, rheumatism, gout, and sciatica (Barros et al., 2010). Galactolipid (GOPO), an active compound found in *Rosa canina*, has been proven effective in treating osteoarthritis (Rutkowska et al., 2012).

Studies have shown that water extracts and active compounds isolated from cistus (*C. incanus* L.) have many valuable properties (Barrajón-Catalán et al., 2010). Infusions of the plant have been used in traditional medicine due to their anti-inflammatory potential. Aqueous and ethanolic extracts are known to exhibit significant antioxidant activity (Gaweł-Bęben et al., 2020). Cistus products are particularly prominent for their high content and diverse profile of polyphenolic substances with potent antioxidant activity (Kuchta et al., 2021).

Moringa oleifera L. is considered one of the most useful trees in the world. It has been recognized by researchers as a plant with numerous health benefits, including nutritional and medicinal. *Moringa olefera* L., due to its rich chemical composition, exhibits many benefits for human health, among which anti-inflammatory, anti-bacterial, anti-cancer, antioxidant and hepatoprotective properties can be listed. Its extract exhibit antioxidant, hypolipidaemic and antiatherosclerotic activities (Chumark et al., 2008).

Thanks to the presence of inulin and fructooligosaccharides, Jerusalem artichoke tubers are an important raw material for the production of functional foods. Consumption of *H. tuberosus* L. tubers regulates blood pressure, lowers the level of “bad cholesterol”, protects the kidneys and liver, and facilitates the absorption of calcium, magnesium, and iron (Sawicka et al., 2018).

Ashwagandha plant is characterized by a very rich chemical composition. Its main active constituents are a group of compounds called withanolides, which are naturally occurring steroidal lactones and alkaloids. The plant is known as a powerful adaptogen, which increases the body’s resistance to stress and has a positive effect on the functioning of the brain and nervous system. In addition, ashwagandha improves memory, and exhibits anti-inflammatory effects (Połumackanycz et al., 2020). Preclinical studies indicate a potential role of ashwagandha as a nootropic agent, promoting cognitive function and improving memory (Choudhary et al., 2017).

The aim of the study was to evaluate the effect of selected plant additives on the physicochemical profile of beer. The study used the brewer’s wort with the addition of aniseed, cistus, rosehip, moringa oleifera, Jerusalem artichoke, and ashwagandha at two doses, i.e., 0.1% and 0.2% (w/w). The beer without any of these ingredients was used as a control sample. Therefore, the total of 13 variants was evaluated (2 variants of each ingredient, and a control sample). The samples were inoculated with *Saccharomyces cerevisiae* Safale US-05 yeast. Changes in samples weight during fermentation were analyzed. The content of alcohol, real extract, free amino nitrogen, as well as titratable acidity, color, and pH were determined in the beers. Additionally, a sensory analysis of the resulting beers was conducted.

MATERIALS AND METHODS

Materials

The wort used for the study was sourced from one of the well-known breweries from Krakow (Poland). Wort parameters: extract 12.1°P; grain bill: pilsner malt 50% and wheat malt 50 %; Marynka hops, bitterness level: 10 IBU.

Yeasts used in the study: *Saccharomyces cerevisiae* Safale US-05.

The following plant ingredients (in a dried natural, not ground form) were used: anise - *Pimpinella anisum* L. (food grade, KOL-POL, Dębica, Poland), rosehip - *Rosa canina* L. (food grade, Herbanordpol-Gdańsk Sp. z o.o., Nowy Staw, Poland), cistus - *Cistus incanus* L. (food grade, KOL-POL, Dębica, Poland), ashwagandha root - *Withania somnifera* L. (food grade, Planteon sp. z o.o., Żelazków, Poland), moringa leaf - *Moringa oleifera* L. (food grade, GREEN ESSENCE, Pyrzyce, Poland), Jerusalem artichoke tubers - *Helianthus tuberosus* L. (food grade, K-Mystery, Seosan-si, Republic of Korea).

Inoculation and fermentation

The direct raw material used for the fermentation process was wort (extract 12.1°P, 10 IBU), and worts with aniseed, cistus, rosehip, oil moringa, Jerusalem artichoke, and ashwagandha. All of the variants used the same wort, and the same volume of it. The only difference was different additions (or lack of them in a control sample). The addition of plant materials was set at 0.1 and 0.2% (w/w). Therefore, the total of 13 variants was evaluated (2 variants of each ingredient, and a control sample). The samples of 0.3 L were fermented in 0.5 L glass flasks.

To prepare each of the variant, 0.3 L of the wort was added to a glass fermentation flask. Then, an appropriate amount (0.1 or 0.2% by weight) of a given ingredient was added, and *Saccharomyces cerevisiae* Safale US-05 yeasts were introduced in an amount of 0.5 g d.w./L. After carefully closing the flasks and attaching fermentation tubes filled with glycerin, the system was additionally sealed with parafilm. The ingredients stayed in the wort through whole fermentation. The fermentation process was conducted for 19 days at the temperature of 20°C (each sample in triplicate).

Methods

Determination of fermentation dynamics

The fermentation rate was determined on the basis of a sample mass loss resulting from CO_2 escaping from the setup. The samples were weighted every 24 h with 0.01 g accuracy. Results from three independent repetitions were presented as a percentage loss of the fermentation media mass.

Determination of real extract and alcohol content

The alcohol concentration in the final beer was determined using the pycnometric method. For this purpose, the sample after fermentation was distilled. The obtained distillate was filled up to 100 g with distilled water, its density was determined, and the concentration of ethanol was read from the adequate tables. The distillation residue was quantitatively transferred to a 100 mL volumetric flask, made up to 100 g with distilled water, and the real extract of the samples was determined (analogous to alcohol), (Analytica EBC Methods 9.2.1, Analytica EBC Methods 9.4), (Analytica EBC, European Brewery Convention, 1998).

The wort extract was determined using a refractometer.

Determination of titratable acidity

The potentiometric method was applied to determine titratable acidity, titrating a sample with 0.1M NaOH solution to obtain pH = 8.

Determination of FAN content

Free amino nitrogen (FAN) was measured using the ninhydrin-based method with the use of the absorbance measurement at 570 nm (Beckman DU-650 UV – Vis) according to the method: 8.10 Free Amino Nitrogen in wort by Spectrophotometry (IM) (Analytica EBC, European Brewery Convention, 1998).

Determination of color

The color of the filtered samples was determined spectrophotometrically (Beckman DU-650 UV-Vis) at a wavelength of 430 nm (according to Analytica EBC Methods 8.5 and Analytica EBC Methods 9.6).

Determination of pH

The determination consisted of direct measurement of the samples using a pH meter (Elmetron CP-505).

Sensory analysis

Sensory analysis of beers consisted of aroma, flavor, clarity, and color evaluation using a descriptive test. Distinctive aromas (including fruity, herbal, spicy, floral, vinegary, yeasty), and colors (including amber, gold, brown, tea, nutty) were evaluated – each according to a 5-point scale.

The beers were judged by an eight-member panel of four women and four men. All panelists were well-acquainted with the principles of the analysis and trained in sensory analysis. The results are presented as an arithmetic mean.

Statistical analysis

The results have been presented as the arithmetic mean of three repetitions, standard deviation included. Additionally, repeated measures ANOVA and a Tukey (HSD) multiple range tests at the significance level of $\alpha = 0.05$ were performed, where applicable.

RESULTS AND DISCUSSION

Characteristics of wort and wort with the addition of plant ingredients

The highest acidity was found in the samples with the addition of ashwagandha (0.2%), and moringa leaves (0.2%) (Table 1). Whereas the lowest value of this parameter was determined in a variant with the addition of cistus (0.2%). It was observed that in nearly all of the wort samples, the addition of plants increased the titratable acidity of a sample, as compared to the control. The exception was the variants to which cistus was added, where the addition of the plant did not affect the acidity of the wort in both cases (0.1 and 0.2%) (Table 1). The increase in titratable acidity is often caused by increased buffering capacity of a solution/beverage (Shenoy et al., 2020). As the wort buffering capacity is mostly influenced by the content of weak organic acids, these plant materials may have supplemented a significant amount of these compounds (Li et al., 2016).

The wort with the addition of aniseed (0.2%) exhibited the highest levels of real extract, while the lowest values of this parameter were observed in the variants containing ashwagandha (0.1%), moringa leaves (0.1%), and the control sample (12.1°P). The addition of plant materials led to a slight increase in the sample's extract levels compared to the control. Furthermore, higher addition of evaluated ingredients resulted in higher levels of the extract (Table 1). However, these differences were relatively small, as the biggest rise in extract level of a wort resulted in only 0.8 °P rise for the aniseed sample (0.2%).

The wort with a rosehip (0.2%) was found to have the darkest color (18 EBC), while the variant with ashwagandha (0.2%) had the lightest color (11.7 EBC).

The free amino nitrogen (FAN) content of the worts ranged from 97.6 mg/L to 127 mg/L. According to various sources, the wort FAN levels should be in the range of 100 – 150 mg/L. Hill and Stewart (Hill and Stewart, 2019) state that the value of this parameter should oscillate at around 130 mg/L. The obtained results show that most of the plant ingredients used caused a significant drop of FAN levels in most of the worts. In a study by Wu et al. (Wu et al., 2024), the addition of tea at the beginning of the fermentation process did not influence the FAN levels, whereas the additions at earlier stages of a process resulted in higher FAN levels (Wu et al., 2024). It therefore seems, that some materials can result in a lower wort free amino nitrogen content, and it should be noted when these materials are used.

Fermentation process

Figure 1 shows the fermentation course of the wort with the addition of plants (at 0.1%), and the control sample. It was observed that for all of the variants, the process proceeded rapidly during the first 6 days (primary fermentation), during which the decreases in samples weight were significant. This is due to the rapid adaptation of the yeasts to the new environmental conditions, and the intensive fermentation of simple sugars contained in the wort. Subsequently, the process slowed down and stabilized in the following days, with weight loss continuing at a consistent but minor level. The weight loss in a control sample was slightly lower than in worts enriched with raw materials used (Figure 1). That suggests, that the plant materials might have supplemented nutrients to help the yeast cells to adapt to the environment. The fermentation of the samples with the addition of plant materials (at 0.2%) was very similar (Figure 2).

As can be seen from Figures (1 and 2), the addition of the selected plants affected the fermentation process. Worts with plant materials were characterized by a more intense fermentation process and a greater decrease in weight compared to the control sample (Figures 1 and 2).

Table 1. Physicochemical characteristics (mean of 3 replicates ± standard deviation) of worts and worts with additives.

Wort with tested additive [%]		Real extract [°P]	Titratable acidity [mL 1M NaOH per 100 mL of wort]	pH	Color [EBC]	FAN [mg/L]
Wort + aniseed	0.1	12.6 c ± (0.00)	1.12 c ± (0.01)	5.67 gh ± (0.00)	15.3 j ± (0.01)	121 h ± (1.20)
	0.2	12.9 d ± (0.00)	1.16 d ± (0.01)	5.67 gh ± (0.00)	15.3 k ± (0.01)	111 e ± (1.30)
Wort + ashwagandha	0.1	12.1 a ± (0.00)	1.12 c ± (0.01)	5.61 de ± (0.01)	14.6 e ± (0.01)	99.8 b ± (2.56)
	0.2	12.5 bc ± (0.00)	1.24 f ± (0.01)	5.57 b ± (0.00)	11.7 a ± (0.00)	101 bc ± (2.10)
Wort + cistus	0.1	12.2 a ± (0.00)	1.04 b ± (0.01)	5.69 h ± (0.01)	14.5 d ± (0.00)	127 i ± (2.00)
	0.2	12.7 cd ± (0.00)	1.00 a ± (0.00)	5.62 de ± (0.01)	12.4 c ± (0.01)	118 g ± (2.60)
Wort + moringa leaves	0.1	12.1 a ± (0.00)	1.12 c ± (0.01)	5.61 de ± (0.00)	16.2 l ± (0.01)	102 d ± (1.02)
	0.2	12.6 c ± (0.00)	1.24 f ± (0.01)	5.60 cd ± (0.01)	14.8 g ± (0.01)	101 cd ± (1.00)
Wort + rosehip fruit	0.1	12.3 ab ± (0.00)	1.20 e ± (0.01)	5.55 b ± (0.00)	14.7 f ± (0.01)	102 d ± (0.58)
	0.2	12.7 cd ± (0.00)	1.20 e ± (0.01)	5.46 a ± (0.00)	18.0 ł ± (0.00)	97.6 a ± (1.65)
Wort + artichoke	0.1	12.6 c ± (0.00)	1.12 c ± (0.01)	5.63 def ± (0.01)	14.9 i ± (0.00)	113 f ± (2.65)
	0.2	12.7 cd ± (0.00)	1.12 c ± (0.01)	5.64 efg ± (0.00)	11.8 b ± (0.01)	111 e ± (3.10)
Control sample	-	12.1 a ± (0.00)	1.00 a ± (0.00)	5.66 fgh ± (0.00)	14.8 h ± (0.01)	127 i ± (1.98)

The obtained values were compared statistically using multivariate analysis of variance – ANOVA. The results of statistical significance correspond to the interaction of all variables. No assigned letters indicate the absence of statistically significant differences in a given group of results at $p<0.05$.

a-ł – mean values marked with different letters (for individual parameters) show statistical variation according to Tukey's test (HSD) ($p<0.05$).

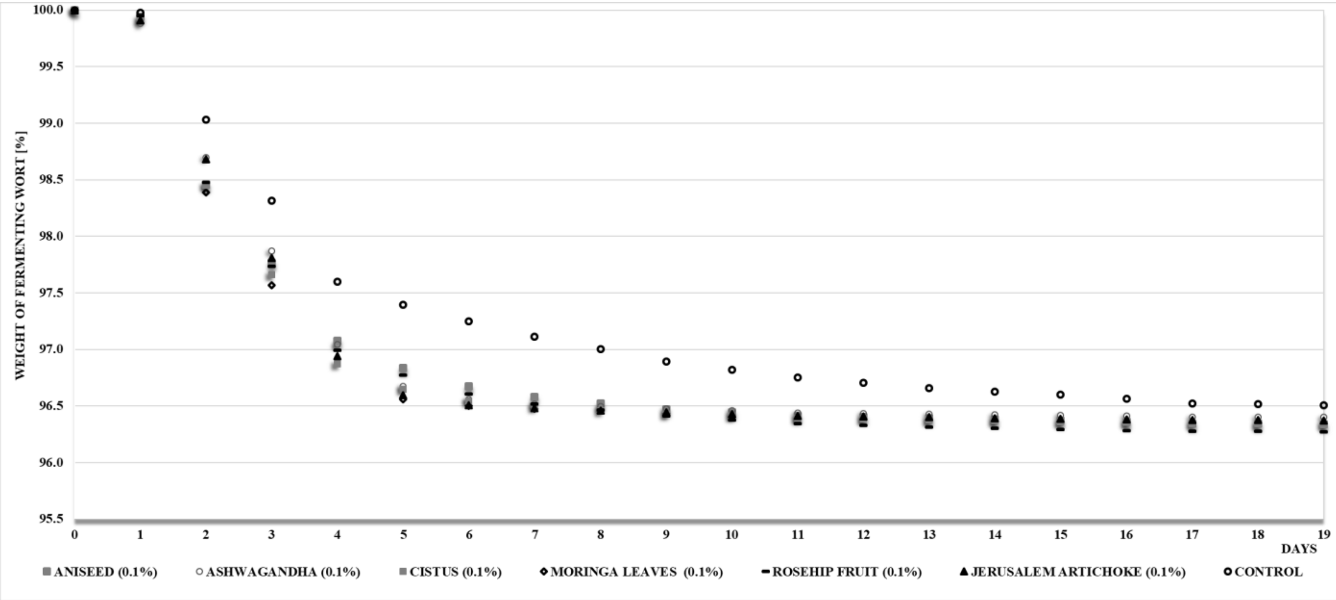

Figure 1 Fermentation dynamics of fermenting worts with plants (0.1%). Standard deviation <0.5%

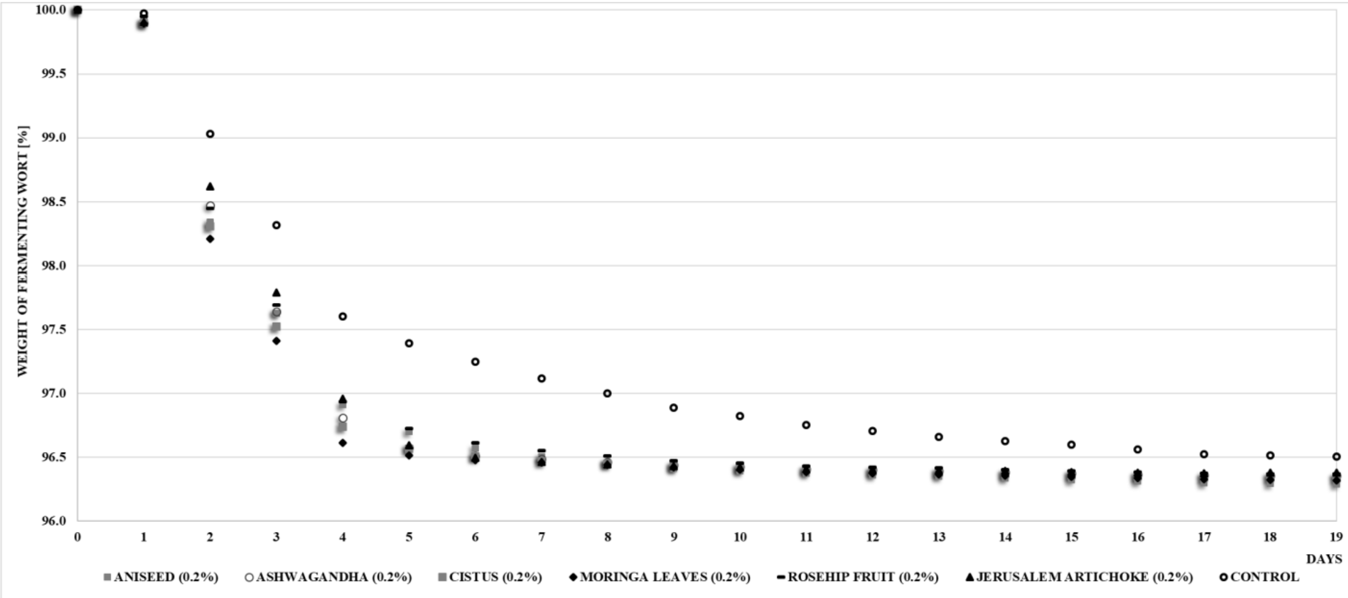

Figure 2 Fermentation dynamics of fermenting worts with plants (0.2%). Standard deviation <0.5%

Physicochemical characteristics of beers

The alcohol and real extract content

The real extract content of the samples ranged from 42.9 g/L to 45.2 g/L (Table 2). Given the amount of ethanol in the beers (5.22% - 5.40% alcohol by volume - ABV), it is reasonable to assume that the high extract content may be due to the high amount of non-fermentable sugars in the wort. Ethanol, along with carbon dioxide, is the main product of the fermentation process. It is also the main alcohol present in the beer by volume. Its content in the beverage depends on the yeast strain used, as well as the fermentation conditions, which affect the growth and proper functioning of the microorganisms. The average strength of beers found on the market is between 4.8% and 5.2% ABV, but the range is much wider. There are many beers that contain as low as 3.5% ABV or even lower, while some drinks can reach 12% ABV (Garrett, 2012). Ethanol, and volatile compounds, especially esters and higher alcohols are known to have a significant impact on the flavor profile and aroma of beer (Olaniran et al., 2017).

The highest ethanol concentration was found in the variant with Jerusalem artichoke (0.2%), while the lowest level was determined in the variant with ashwagandha (0.2%), with 5.40% ABV, and 5.22% ABV, respectively (Table 2).

Table 2. Physicochemical characteristics of beers (mean of 3 replicates ± standard deviation).

Beer with tested additive [%]		Real extract [g/L]	Alcohol [% vol.]	pH	Titratable acidity [mL 1M NaOH per 100 mL of beer]	Color [EBC]	FAN [mg/L]
Beer + aniseed	0.1	44.2 ± (0.86)	5.29 ± (0.02)	4.25 ± (0.05)	2.81 ± (0.13)	12.3 ab ± (0.83)	45.9 c ± (4.34)
	0.2	43.7 ± (0.00)	5.39 ± (0.00)	4.25 ± (0.03)	2.92 ± (0.20)	12.3 ab ± (1.04)	59.4 e ± (5.18)
Beer + ashwagandha	0.1	43.9 ± (0.43)	5.27 ± (0.07)	4.13 ± (0.03)	3.04 ± (0.14)	12.0 ab ± (0.73)	36.8 b ± (1.01)
	0.2	44.2 ± (0.19)	5.22 ± (0.01)	4.08 ± (0.03)	3.17 ± (0.13)	10.2 a ± (1.20)	28.5 a ± (0.05)
Beer + cistus	0.1	43.7 ± (0.71)	5.30 ± (0.05)	4.13 ± (0.02)	3.05 ± (0.06)	12.0 ab ± (0.16)	56.7 de ± (1.39)
	0.2	43.7 ± (0.20)	5.36 ± (0.03)	4.15 ± (0.02)	3.13 ± (0.06)	10.8 a ± (1.59)	50.2 cd ± (4.34)
Beer + moringa	0.1	43.1 ± (0.24)	5.37 ± (0.03)	4.15 ± (0.03)	3.13 ± (0.02)	13.4 ab ± (0.49)	45.3 c ± (0.29)
	0.2	42.9 ± (0.23)	5.38 ± (0.04)	4.17 ± (0.01)	3.25 ± (0.06)	12.9 ab ± (1.34)	63.6 e ± (1.38)
Beer + rosehip fruit	0.1	44.7 ± (0.19)	5.23 ± (0.03)	4.12 ± (0.04)	3.04 ± (0.04)	11.6 ab ± (1.99)	31.6 ab ± (0.93)
	0.2	45.2 ± (0.13)	5.26 ± (0.00)	4.15 ± (0.01)	3.07 ± (0.08)	15.7 b ± (1.70)	45.2 c ± (0.41)
Beer + Jerusalem artichoke	0.1	44.1 ± (0.40)	5.35 ± (0.01)	4.15 ± (0.00)	3.04 ± (0.07)	12.3 ab ± (1.33)	59.8 e ± (1.73)
	0.2	44.7 ± (0.21)	5.40 ± (0.03)	4.19 ± (0.01)	3.04 ± (0.12)	13.3 ab ± (0.18)	58.8 e ± (0.38)
Control sample	-	45.1 ± (0.95)	5.34 ± (0.09)	3.41 ± (0.17)	3.41 ± (0.17)	12.4 ab ± (1.17)	49.1 c ± (2.53)

The obtained values were compared statistically using multivariate analysis of variance – ANOVA. The results of statistical significance correspond to the interaction of all variables. No assigned letters indicate the absence of statistically significant differences in a given group of results at $p<0.05$.

a-e – mean values marked with different letters (for individual parameters) show statistical variation according to Tukey's test (HSD) ($p<0.05$).

Jerusalem artichoke flour is rich in, among other things, inulin (over 50% dry matter), which is easily hydrolyzed and converted into ethanol. The ethanol yield of that flour is equal to that of cane sugar, and twice that of maize (Ciocan et al., 2020). However, the weights of the ingredients used (0.1 and 0.2%) were not high enough to substantially influence the alcohol content of the resulting beers. It must also be kept in mind that some of the plant additives are known for their antagonistic properties against microorganisms, particularly yeasts (for example aniseed (Kubo et al., 2008) or moringa (Patel et al., 2014)). Hence, they might negatively affect the course of the fermentation and/or resulting alcohol levels. On the other hand, other materials might positively affect fermentation course (for example *Chlorella vulgaris* extract (Okechukwu et al., 2024)), supplying nutrients or fermentable sugars. The studied ingredients did not seem to negatively affect the resulting alcohol levels

The resulting beers can be categorized as full beers, as each of them contained at least 5% ABV. The beers with 0.2% of plant ingredients, with the exception of ashwagandha, were characterized by higher ethanol levels, compared to the 0.1% counterparts (Table 2). The relatively high alcohol content of the resulting beers was probably due to the efficient utilization of nutrients occurring in the wort by yeasts. The high ethanol tolerance makes *S. cerevisiae* one of the most important yeast species used in brewing (Mulero-Cerezo et al., 2019). Tozetto et al. (Tozetto et al., 2019) obtained a pilsner beer with the addition of ginger (*Zingiber officinale*), which contained 3.4% ABV. Whereas the beverages produced with the addition of green tea were characterized by an alcohol content in the range of 4.17%-4.29% (Yang et al., 2023). The beer, brewed by Ciocian et al. (Ciocan et al., 2020) using acorn flour, Jerusalem artichoke flour, and molasses contained between 7.06% and 8.16% ABV.

Titratable acidity and pH

The organic acids present in beer do not only bestow it with a sour taste. Some of them are also characterized by other taste attributes, such as bitter or salty. The main source of these compounds in wort is malt. The most abundant acids in wort are citric, lactic, malic, acetic, and formic. The quality of the barley and the malting conditions are mainly responsible for the variation in the organic acid content of different malts. Beer, unlike wort, is characterized by a higher content of organic acids, which is mainly attributed to the production of succinic, lactic, and acetic acids by the yeasts during the fermentation process. The increased acidity of the fermented wort results in a lower pH. The degree of the pH drop during the fermentation process significantly affects the final quality of the beer by changing many of its attributes, such as taste (Li and Liu, 2015).

The titratable acidity of the beers with the addition of plant ingredients ranged from 2.81 mL 1M NaOH per 100 mL for the sample with aniseed fruit (0.1%), to 3.25 mL 1M NaOH per 100 mL for the beer with moringa leaves (0.2%) (Table 2). The highest acidity was found in the control sample, with 3.41 mL of 1M NaOH per 100 mL of beer. The lowest values of this parameter were found for beers with aniseed fruit (in both variants). It was observed that the acidity of the final product rose with an increased amount of plant material in the worts. The exception was the beer with the Jerusalem artichoke – in both variants, the titratable acidity was 3.04 mL of 1M NaOH per 100 mL of beer (Table 2). This is an inverse result as compared to the results for the wort acidity. Whereas in worts, the supplementation of plant material generally resulted in increased acidity, in case of the resulting beers, the acidity was lower. This could be due to the less acidic, or mildly alkaline pH of materials used. The juice of Jerusalem architoke is reported to have pH of 6.5 (Kosaric et al., 1984). Similar fall in titratable acidity is observed when dry-hopping is applied (Monacci et al., 2024).

The acidity of the beer arises mainly from the content of citric and lactic acids. Titratable acidity can increase as a result of infection with lactic acid bacteria, high CO_2 saturation, and a significant amount of acidulated malt in the grain bill. Acetic acid, which is desirable in lambics and flanders styles, is present in greater quantities in sour beers. Excessively high concentrations of acetic acid can indicate potential contamination of the beverage with *Acetobacter* bacteria (Michalski et al., 2018).

Of the beers enriched with plant ingredients, the highest pH levels were found in the samples enriched with aniseed fruit (0.1% and 0.2%, 4.25), while the lowest was determined in the beverage with ashwagandha (0.2%, 4.08). The pH of the control sample was the lowest at 3.41 (Table 2). Similar values were obtained by Pinto et al. (Pinto et al., 2015) for a craft beer brewed with the addition of acerola and pineapple (4.24). The literature sources state that the pH of craft beers can reach lower values, compared to beers commonly found in the market. In a study by Matsubara et al. (Matsubara and Plath, 2014) authors produced an alcoholic beverage with the addition of ginger, the pH levels of which were in the range of 4.13-4.27.

Color

The color ranged from 10.2 EBC for the beer with ashwagandha (0.2%), to 15.7 EBC for the sample with rosehip (0.2%). Increasing the amount of rosehip and Jerusalem artichoke (0.2%) resulted in a darker color than the respective variants with 0.1% of these plants (Table 2). The darker color of the beer with the addition of rosehip can be at least partially attributed to a significant content of tannins, flavonoids, and carotenoids present in this fruit. This plant also contains a significant amounts of lycopene and carotenes. These compounds are known for their health benefits. Materials such as fruits, plants, or spices often contain a substantial amount of color-active compounds. According to the literature, if these materials are used in craft beer production, these compounds might get extracted and significantly affect the color of the resulting beer. For example, the main compounds determining the color of fruits such as blackcurrant and cherries are anthocyanins, while lemon and grapefruit contain naringin, hesperidin, and eriocitrin (Koren et al., 2020). These color-active compounds, apart from bestowing a beer with an interesting properties, have also many health benefits. It was observed that the color of the resulting beers, including the control one, was lighter by about 2 EBC units, compared to the worts with or without evaluated plant materials (Table 1). This is related to the lowering of pH during the fermentation process, as well as the absorption of color-active compounds on the yeast cells' surface, their precipitation, and accumulation in the lees and foam (Kunze, 1999).

Free Amino Nitrogen content

Wort is a growth medium that contains, among other components, fermentable sugars (fructose, sucrose, maltose, and maltotriose), nitrogenous compounds (amino acids, peptides, proteins), vitamins, ions, and minerals. Nitrogenous compounds make up 3-5% of the wort extract, of which about 30% is α-amino nitrogen, 20% is high-molecular-weight proteins and 40% is polypeptides. Nitrogen contained in the purines and other nitrogenous substances makes up the remaining 10% (Ferreira and Guido, 2018). FAN is a measure of nitrogenous compounds, which can be assimilated or metabolized by yeasts during fermentation. It is estimated that up to 70% of the wort's FAN is formed during the malting process (Hill & Stewart, 2019). The amino acids and simple peptides present in the malt provide an important nutrition source (assimilable nitrogen) for the yeasts and are responsible for their growth. Therefore, a sufficiently high FAN content in the malt, and subsequently in the wort, influences the correctness of the fermentation process. The proper course of the fermentation process is an important step in obtaining the desired organoleptic parameters of the beer (Michałowska, 2017; Kucharczyk et al., 2018). Brewer's yeast can assimilate approximately 50% of the wort's amino nitrogen. An important distinction between the yeasts is the fact that bottom-fermenting strains utilize amino acids to a lesser extent than top-fermenting strains (Ferreira and Guido, 2018).

The obtained results (Table 2) show that the FAN content of the obtained beers varied between 28.5-63.6 mg/L. The variant with moringa leaves (0.2%) was characterized by the highest FAN amount, while the beverage with ashwagandha (0.2%) had the lowest. As regards the moringa leaves samples, a substantial difference in FAN levels, amounting to 18.3 mg/L was observed between the 0.1% and 0.2% variants. However, the initial drop of the FAN caused by the addition of plant material disappeared in most of the variants. This suggests, that the previously observed losses of FAN might have been caused by some specific adsorption of FAN into plant material. The FAN diffused from the plant material, as it's levels fell during the fermentation process.

To achieve adequate yeasts growth and fermentation performance, it is estimated that the amount of FAN in the wort should be around 130 mg/L. The minimal levels are cited to be in the range of 100-140 mg/L. Low FAN levels (below 100 mg/L) make the yeasts growth dependent on nitrogen concentration, and its deficiency is associated with delayed and incomplete fermentation (Hill and Stewart, 2019).

The results regarding the FAN levels in the wort and beers attest to a properly conducted fermentation process. The amount of free amino nitrogen in the final product was lower than in the samples before fermentation (Tables 1 and 2). A low FAN amount in beer is desirable, as it slows down the aging processes, and significantly affects the stability of the beverage (Kucharczyk et al., 2018).

Sensory analysis

The primary attributes of a beverage, such as beer, consist of basic sensory characteristics such as taste, aroma, saturation, bitterness, foaminess, clarity, and color (Lutosławski et al., 2018).

In the study, beers obtained with 0.1%, and 0.2% of plant additions, and a control variant without any addition were evaluated regarding their aroma, flavor, color, and clarity. Figures 3 and 4 show the aroma profile of the beers. In the control sample (not enriched with any of the evaluated plant ingredients), the notes that stood out the most (such as yeasty, hoppy, bitter, as well as floral, green apple, and herbal) were typical for a beer. Such an aroma profile may have been influenced by the chemical composition of hops used in brewing the wort. The Marynka variety (bittering hops) which was used to obtain a wort is attributed to have the notes of spice, floral, citrus, and pine. Aromatic compounds and sesquiterpenes derived from hops have a decisive influence on the aroma of a beer. Among the most important compounds, myrcene (the smell of moss or musk), limonene (the smell of tropical fruits, citrus), geraniol, humulene, and linalool (floral notes) can be listed. Sesquiterpenes can bestow a resinous odor, along with spicy, and bitter taste. There are many hopping methods that allow to obtain the desired aroma characteristics. These methods include dry hopping and the utilization of multiple hop varieties either simultaneously or at different stages of the hopping process (Lutosławski et al., 2018).

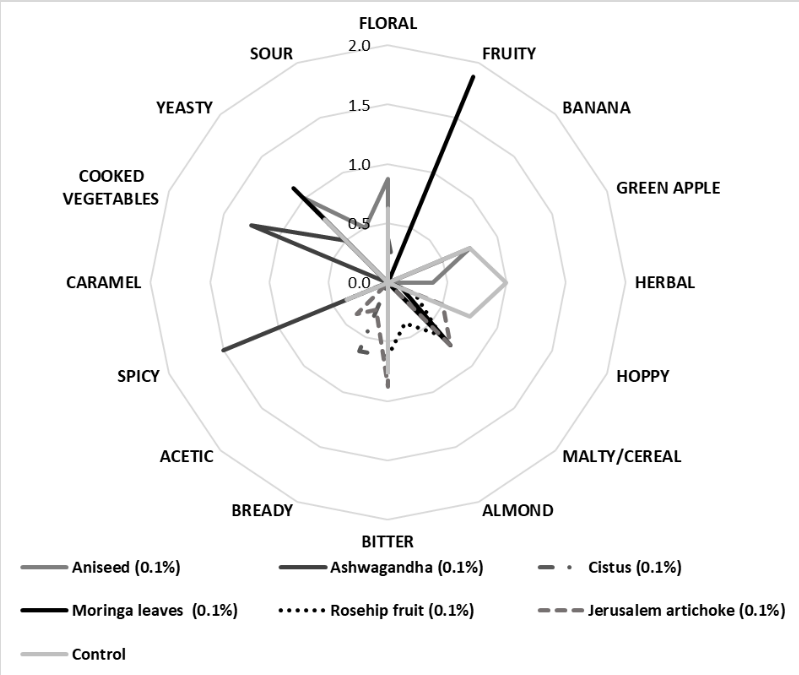

Figure 3 Dominant aromas in beers (addition of the tested plant (0.1%))

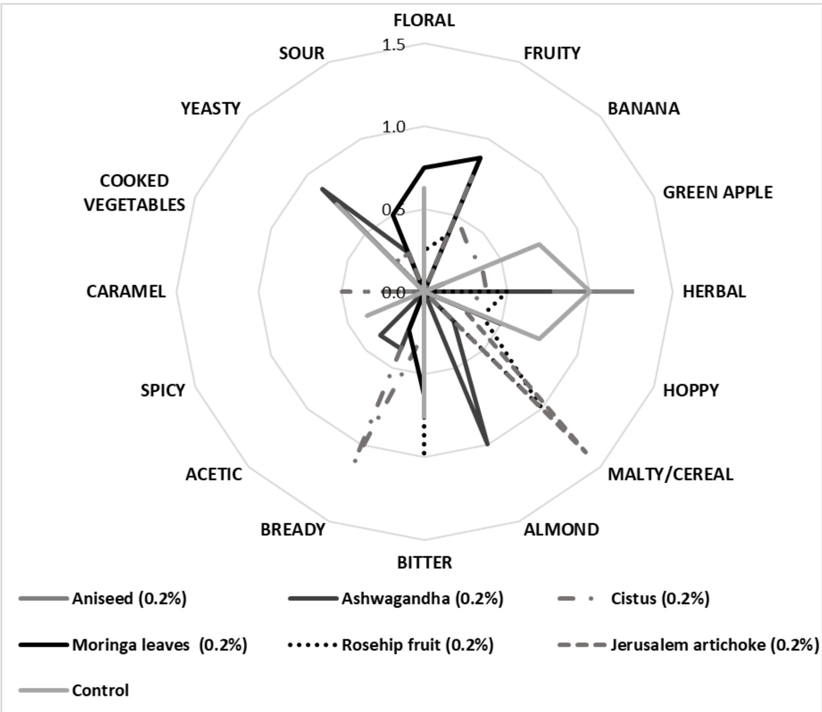

Figure 4 Dominant aromas in beers (addition of the tested plant (0.2%))

The beverages with cistus (in both variants) had the lowest aroma intensity, as compared to the other beers (Figures 3 and 4). The reason for the beer's low aroma rating could be the presence of acetaldehyde, diacetyl, hydrogen sulfide, or sulfur dioxide in the beer (Lutosławski et al., 2018). For example, diacetyl is responsible for the buttery aroma in beer, while acetaldehyde is associated with the smell described as similar to solvent or green apple (Michalski et al., 2018). Interestingly, in 0.2% cistus sample, a strong bready aroma was noticed by the panelists. Cistus at 0.2% was found to have a strong herbal aroma. Rosehip at this dose stood out with bitter aroma. Beers with the addition of moringa leaves (in both variants) were the most aromatic of all variants. They were characterized by floral, fruity, hoppy, yeasty, bitter, sour, and malty aromas. A very strong fruity, and moderate yeast aromas were found in sample with 0.1% addition of this material. Interestingly, the fruity aroma was less pronounced in a beer with higher dose of this ingredient.

Beers with ashwagandha at 0.1% were characterized by a strong spicy and cooked vegetables aroma. Cooked vegetable aroma could have been caused by excessive dimethyl sulfide levels (Bamforth, 2014). When 0.2% addition was evaluated, almond aroma stood out the most.

In beers with Jerusalem artichoke, evaluators indicated the presence of malt, hop, bitter, yeast, fruit, sour, and green apple aromas, although their intensity was lower compared to moringa leaves variants. The aroma which stood out the most in Jerusalem artichoke samples was malty aroma, which was much more pronounced in 0.2% sample (Figures 3 and 4).

Figure 5 presents the results of color, clarity, taste, and overall impression for all of the beers and the control sample.

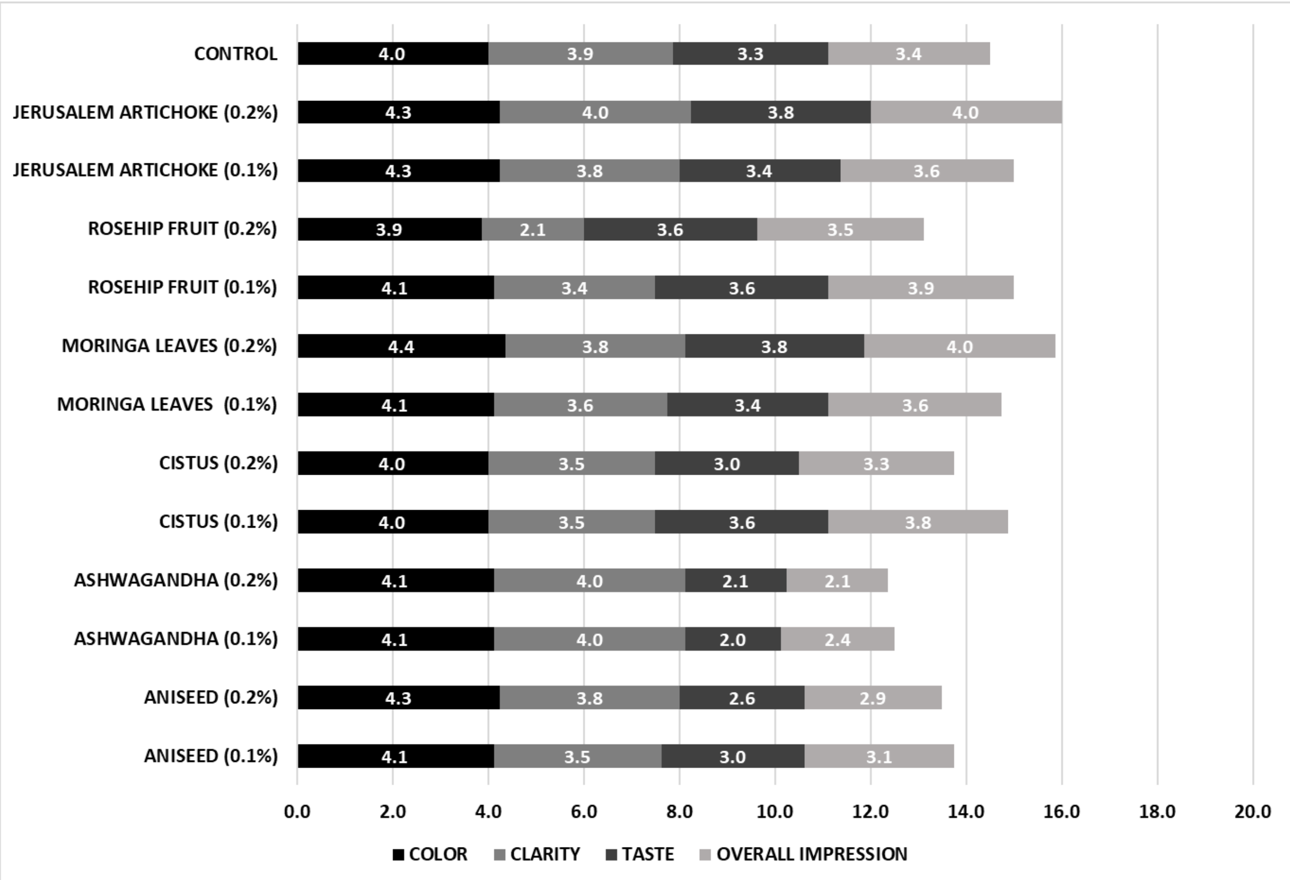

Figure 5 Evaluation of tested beers

In terms of color, the beverage with moringa leaves (0.2%) stood out significantly from the other beer variants and received the highest score (4.4 points). Regarding clarity, beer with rosehip (0.2%, 2.10 points) was found to have the lowest clarity, while in the other evaluated beverages, this attribute was rated at 3-4 points (Figure 5). It must be noted, that some of the plant materials may have an detrimental effect on some beer parameters. For example, olive leaves are known to cause beer haze (Guglielmotti et al., 2020).

The varying aroma intensity of the beers was also reflected in the assessment of taste. This attribute was rated best in the beverage with moringa leaves (0.2%) and with Jerusalem artichoke (0.2%, 3.8

points). The beer with ashwagandha in both variants (Figure 5), was received the worst by the panelists (2 points). Based on the results of the sensory analysis, it can be concluded that the highest sensory quality rating was given to the alcoholic beverages enriched with Jerusalem artichoke (0.2%), and with the addition of moringa leaves (0.2%).

CONCLUSION

Among the tested samples, the highest alcohol content was obtained in the beer with Jerusalem artichoke (0.2%), while the lowest was found in the variant with ashwagandha (0.2%). The beers containing plant ingredients showed reduced titratable acidity compared to the control sample. As a result of the sensory analysis, beers with Jerusalem artichoke (0.2%) and moringa leaves (0.2%) scored best. The dominant aromas in these beverages were floral, fruity, hoppy, yeasty, bitter, sour, and malty.

DECLARATIONS

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